

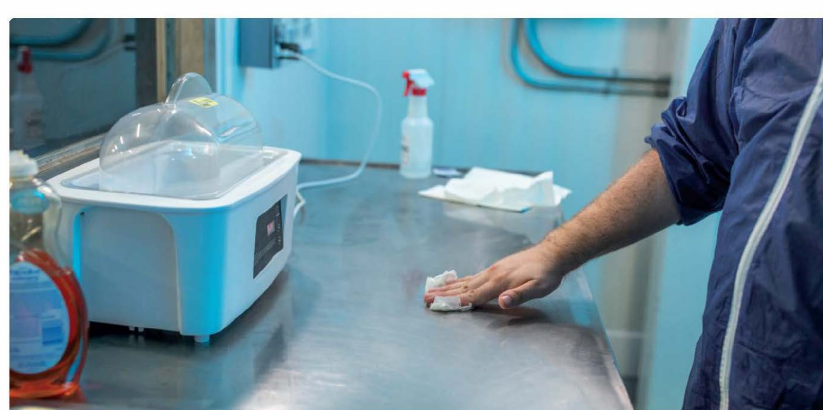
Protocols for preparing frozen Marek's disease vaccines for *in ovo* administration

Vaccines are essential tools for protecting against Marek's disease. Follow these best practices for preparing frozen vaccines shipped and stored in liquid nitrogen.

Always follow label directions, especially when using these frozen vaccines from Zoetis: Poulvac® Ovoline® CVI, Poulvac Marek CVI + HVT, Poulvac Procerta® HVT-IBD-ND, Poulvac Procerta HVT-IBD and Poulvac Procerta HVT-ND.

Attention: Liquid Nitrogen Safety

- Store frozen vaccines in liquid nitrogen until ready for use.
- Check liquid nitrogen levels daily. Contact your supplier if nitrogen levels drop below 3 inches (7.5 cm).
- Wear eye protection and insulated gloves.
- Avoid inhaling liquid nitrogen vapor when checking levels daily.



Step 1: Selecting and Preparing Vaccine Room

Considerations when selecting a vaccination room:

- One that is separated from unclean hatchery areas.
- Space that experiences minimal traffic and is not used for storage.
- Pressure is positive and temperature is constant, about 65 degrees.
- Airflow is not directed at the vaccine preparation surface area.
- Ventilation filters are cleaned or replaced monthly.

Preparing the Surface

- With clean, sanitized hands, disinfect a nonporous working surface with alcohol before getting started.
- Disinfect between the mixing of each bag of vaccine.
- If applying non-Marek's vaccines for *in ovo* application, use a separate, designated area that is not shared with day-of-age vaccination. Maintain separate syringes, dyes, antimicrobial bottles and thaw baths to prepare vaccines and help avoid cross-contamination.



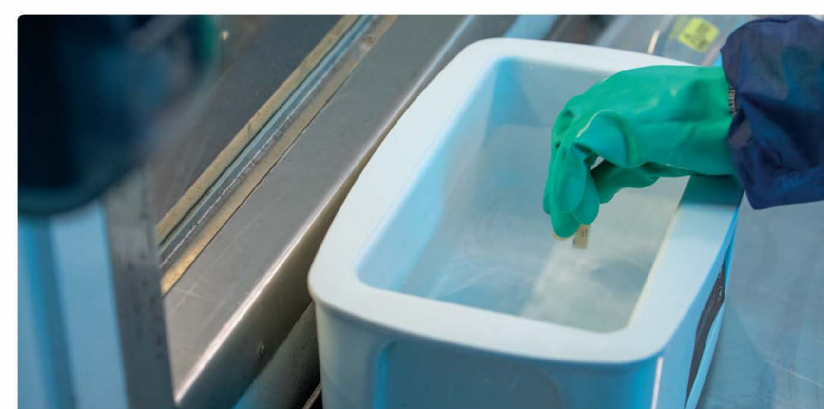
Step 2: Handling Diluent Additives

Preparation

- Use diluents at room temperature.
- Follow manufacturer recommendations for vaccine use, as well as guidance by your veterinarian.

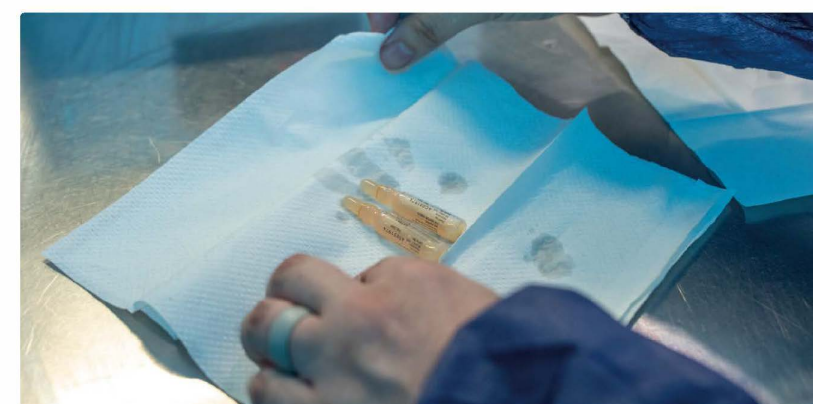
Handling

- Use only clear diluents when preparing vaccine. Discard cloudy or expired diluents. If a diluent appears damaged, stop using it immediately and notify your supplier.
- Use a separate sterile needle and syringe for each product added to the diluent.
- Wipe the diluent bag port with alcohol; allow the surface to dry for 5 seconds before inserting the needle.
- Add dye to the diluent followed by other additives including antibiotic, if used. Then add additional vaccines, with Marek's vaccine always added last.



Step 3: Thawing Vaccine

- Wash the thaw bath with soap and water daily, then dry and spray it down with alcohol.
- Fill the thaw bath with distilled water and keep water temperature at 80° F/27° C. Use a back-up thermometer to confirm temperature.
- Add chlorine to the thaw bath for a final concentration of 200 ppm (15 mL of 5% bleach or sodium hypochlorite per gallon of water; 4 mL per liter of water).
- Pull vaccine ampule cane out of the liquid nitrogen container only as far as needed.
- Place vaccine ampules in thaw bath. Thaw for 70 to 90 seconds — time begins when the ampule is removed from the liquid nitrogen container. Place no more than four ampules in thaw bath at a time. Using a slotted spoon, slowly swirl ampules while thawing.
- Total timespan of vaccine ampule removal from liquid nitrogen, thawing and adding to diluent should be no more than 3 minutes.



Step 4: Opening Ampules

- Dry hands and ampule(s) with a clean paper towel. Use a new paper towel with each thawing sequence of four or fewer ampules.
- Point top of the ampule away from face and body, then carefully break the ampule at the neck.
- Do not spill ampule contents.



Step 5: Extracting Vaccine

- Use a separate sterile syringe and needle for each product added to the diluent.
- Wipe the diluent bag port with alcohol and allow to dry for 5 seconds.
- Draw 5 mL of diluent into a 20-mL sterile syringe with an 18-gauge needle that is 1-1/2 inches long.
- Slowly draw contents of the thawed ampules into the syringe containing diluent. **Caution:** Fast drawing or injecting can cause cell damage to the vaccine and reduce potency.

Recommended: Use a clean ampule holder to reduce the risk of self-injection and help ensure a more stable platform.



Step 6: Mixing Vaccine With Diluents and Injecting

- Slowly add syringe contents to the bag of diluent once vaccine has been extracted.
- On each bag, write the time vaccine is added with a permanent marker.
- Mix contents of the diluent container thoroughly by massaging and inverting the bag about 10 times.
- Do not shake vigorously and avoid creating bubbles.
- The vaccine is now ready for use.



Step 7: Delivering Ready-to-Use Vaccine

- After mixing, place the vaccine in a small cooler with clean plastic-covered ice packs. Keep vaccine inside the cooler until ready to hang in device.
- Dry the outside of bag thoroughly with a new, clean paper towel before hanging.
- This step should be completed by a designated "clean" staff member: Place the vaccine in the Intertherm bag with ice packs. Remove the IV port cover, swab the spike with an alcohol swab. Without twisting, insert spike into the port.
- Return the transport cooler to a designated location in the lab.

IMPORTANT

- Vaccine must be used within 90 minutes from the time it was prepared. Recommendations may differ according to vaccine type and manufacturer.
- While vaccinating, maintain diluted vaccine at 70° F to 80° F.
- Keep a daily vaccine log recording the following:
 - Vaccine types
 - Lot numbers
 - Expiration dates
 - Bag sizes
 - Vaccine amounts
 - Other additives used per bag

For training and audit support on proper *in ovo* vaccination protocols, contact your Zoetis representative to learn more about opportunities to help improve *in ovo* vaccination performance.